



# 人ADARB1与RNA的相互作用关系

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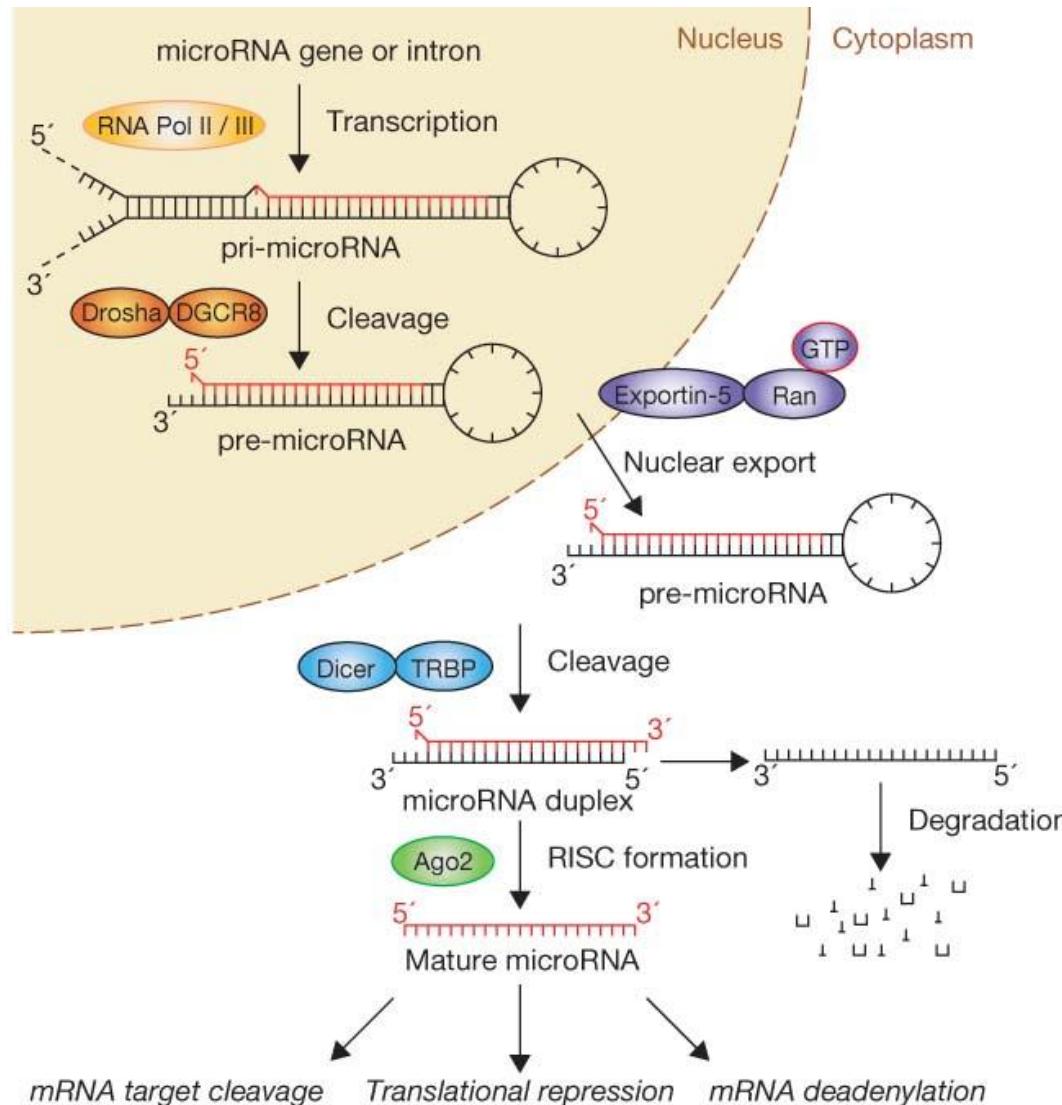
分子医学研究所  
干细胞与非编码核酸实验室

# Outline

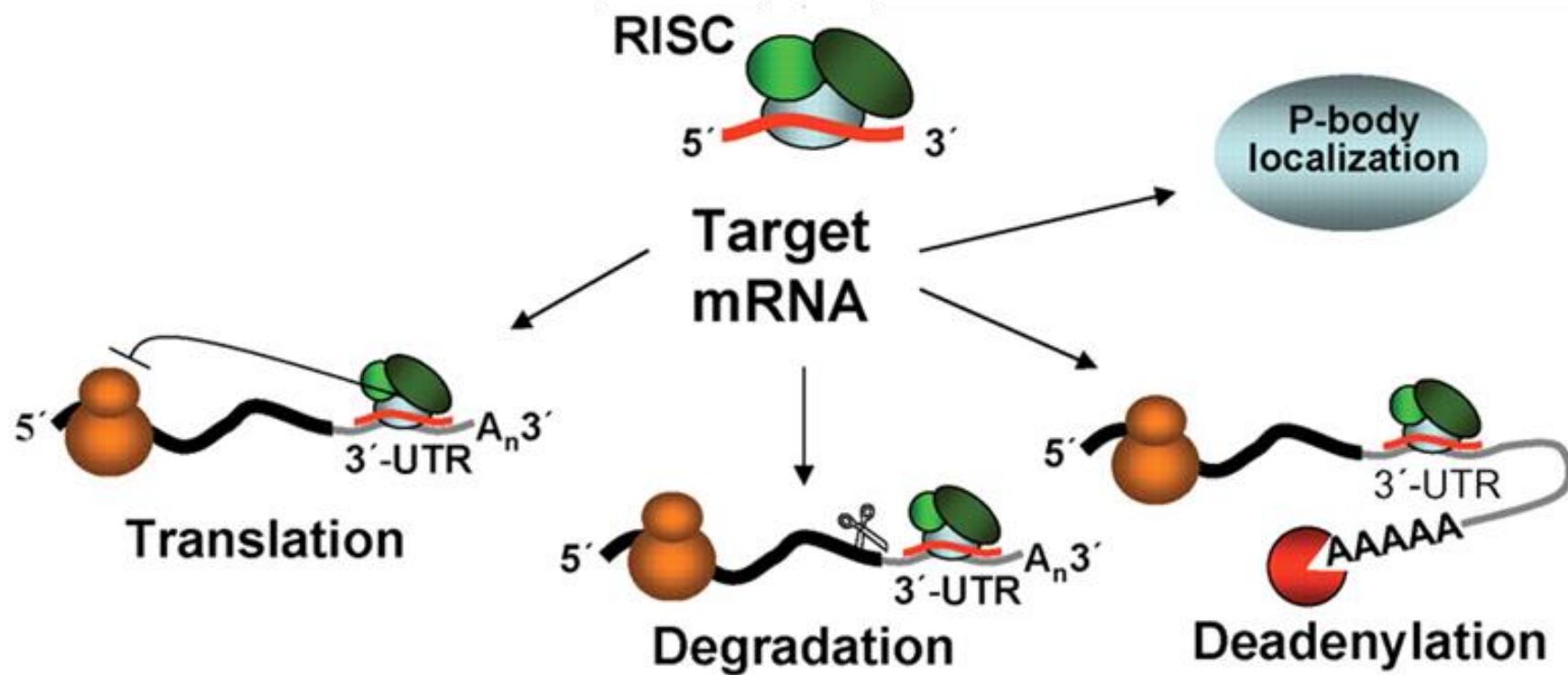
- 课题背景
  - microRNA
  - RNA editing
- 课题内容介绍
  - 研究对象和研究目的
  - 利用生物信息学工具的初步分析
- 小结与讨论

# 课题背景

## microRNA Processing

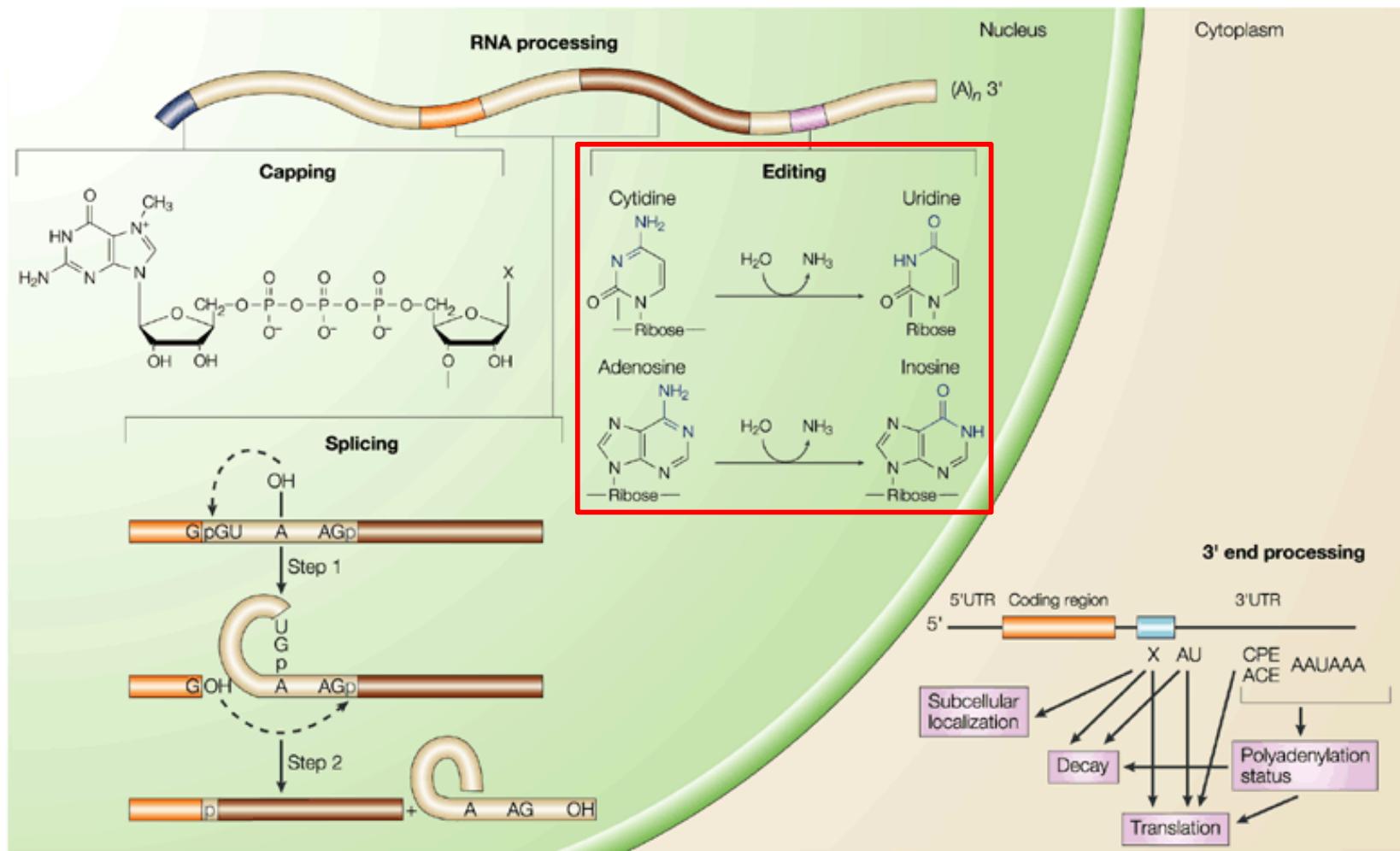


## microRNA targeting



Cardiovasc Res. 2008 Sep 1;79(4):553-61.

# RNA editing



Nature Reviews | Genetics

Nature Reviews Genetics 2, 869-878

# 研究对象和研究目的

ADARB1 may play a role in microRNA processing and regulation

## Names and origin

### Protein names

*Recommended name:*

Double-stranded RNA-specific editase 1  
EC=3.5.4.37

*Alternative name(s):*

RNA-editing deaminase 1  
RNA-editing enzyme 1  
dsRNA adenosine deaminase

### Gene names

Name: **ADARB1**

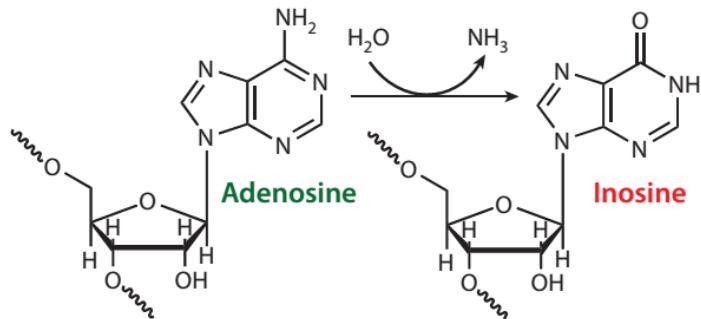
Synonyms: ADAR2, DRADA2, RED1

### Organism

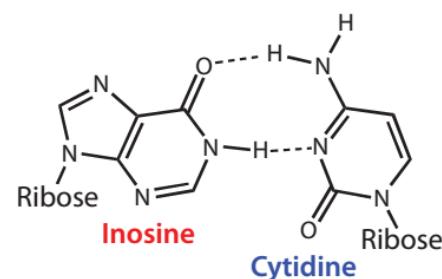
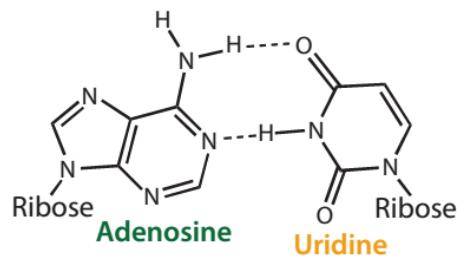
**Homo sapiens (Human)** [Reference proteome]

# ADARB1

a



b



# Uniprot

6 results for domain:"A to I editase domain" AND organism:"Homo sapiens (Human) [9606]" in UniProtKB sorted by score descending

Download

Browse by taxonomy, keyword, gene ontology, enzyme class or pathway |

Reduce sequence redundancy to 100%, 90% or 50%

Page 1 of 1

Results Customize

› Expand search to "Homo sapiens (Human) [9606]" to include lower taxonomic ranks

› Add columns: Domains

Entry	Entry name	Status	Protein names	Gene names	Organism	Length
<input type="checkbox"/> Q9BUB4	ADAT1_HUMAN	★	tRNA-specific adenosine deaminase 1 (hADAT1) (EC 3.5.4.34) (tRNA-specific adenosine-37 deaminase)	ADAT1	Homo sapiens (Human)	502
<input type="checkbox"/> P55265	DSRAD_HUMAN	★	Double-stranded RNA-specific adenosine deaminase (DRADA) (EC 3.5.4.37) (136 kDa double-stranded RNA-binding protein) (p136) (Interferon-inducible protein 4) (IFI-4) (K88DSRBP)	ADAR ADAR1 DSRAD G1P1 IFI4	Homo sapiens (Human)	1,226
<input type="checkbox"/> P78563	RED1_HUMAN	★	Double-stranded RNA-specific editase 1 (EC 3.5.4.37) (RNA-editing deaminase 1) (RNA-editing enzyme 1) (dsRNA adenosine deaminase)	ADARB1 ADAR2 DRADA2 RED1	Homo sapiens (Human)	741
<input type="checkbox"/> Q9NS39	RED2_HUMAN	★	Double-stranded RNA-specific editase B2 (EC 3.5.--) (RNA-dependent adenosine deaminase 3) (RNA-editing deaminase 2) (RNA-editing enzyme 2) (dsRNA adenosine deaminase B2)	ADARB2 ADAR3 RED2	Homo sapiens (Human)	739
<input type="checkbox"/> Q96M93	ADAD1_HUMAN	★	Adenosine deaminase domain-containing protein 1 (Testis nuclear RNA-binding protein)	ADAD1 TENR	Homo sapiens (Human)	576
<input type="checkbox"/> Q8NCV1	ADAD2_HUMAN	★	Adenosine deaminase domain-containing protein 2 (Testis nuclear RNA-binding protein-like)	ADAD2 TENRL	Homo sapiens (Human)	583

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## ADAR蛋白的功能：

1.很多研究表明ADAR蛋白之间会形成同源或异源二聚体，共同发挥作用。

2. 改变氨基酸序列，影响蛋白功能（多发生在中枢神经系统）

GluR, 5-HT2C, Kv1.1, GABAA

3.改变非编码核酸序列，影响转录后切割。

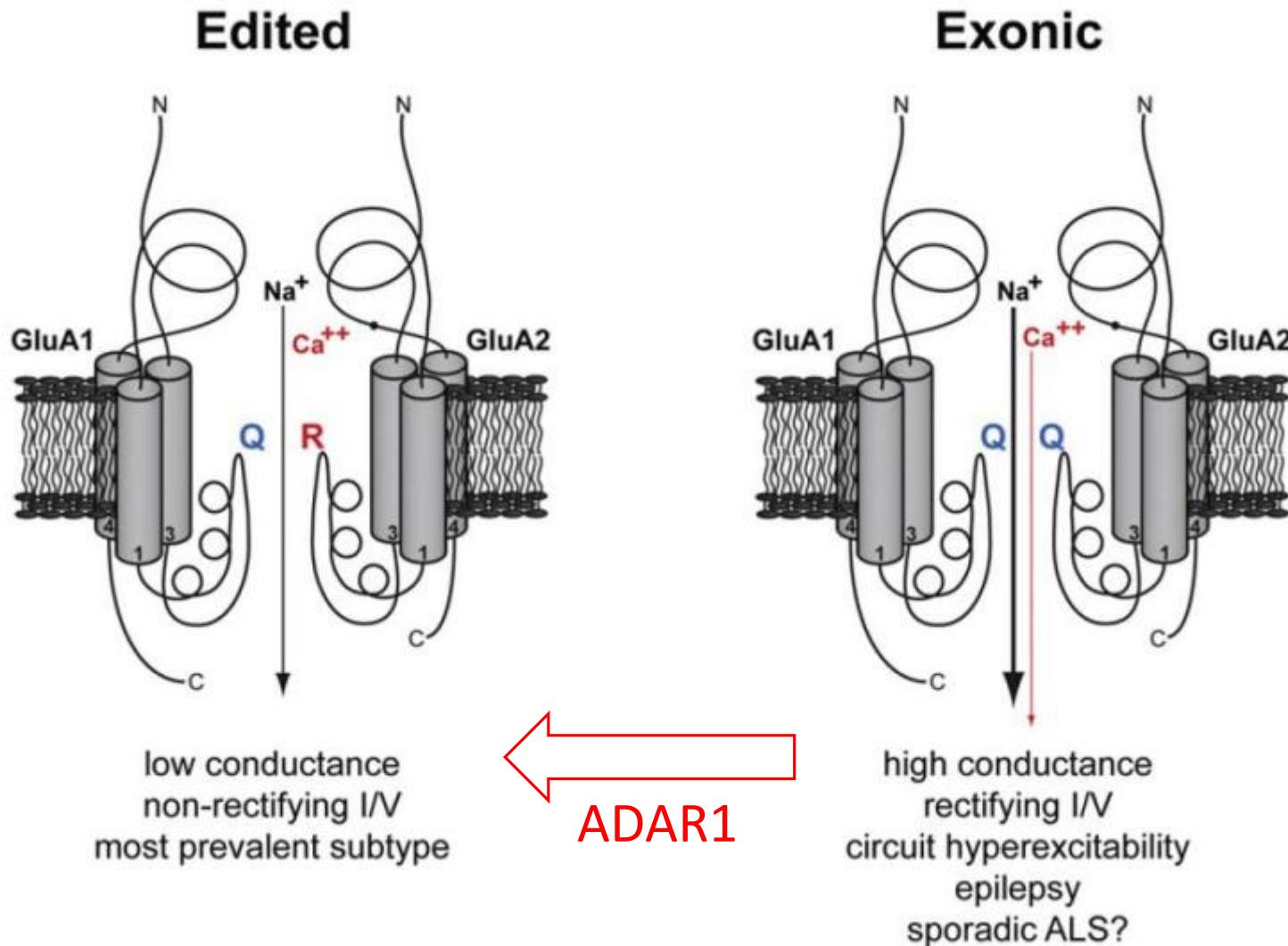
Pri-miR-142, pre-miR-151

4.修饰病毒RNA,影响病毒复制。 ADAR1会被Interferon- $\beta$ 诱导，又被称为Interferon-inducible protein 4。

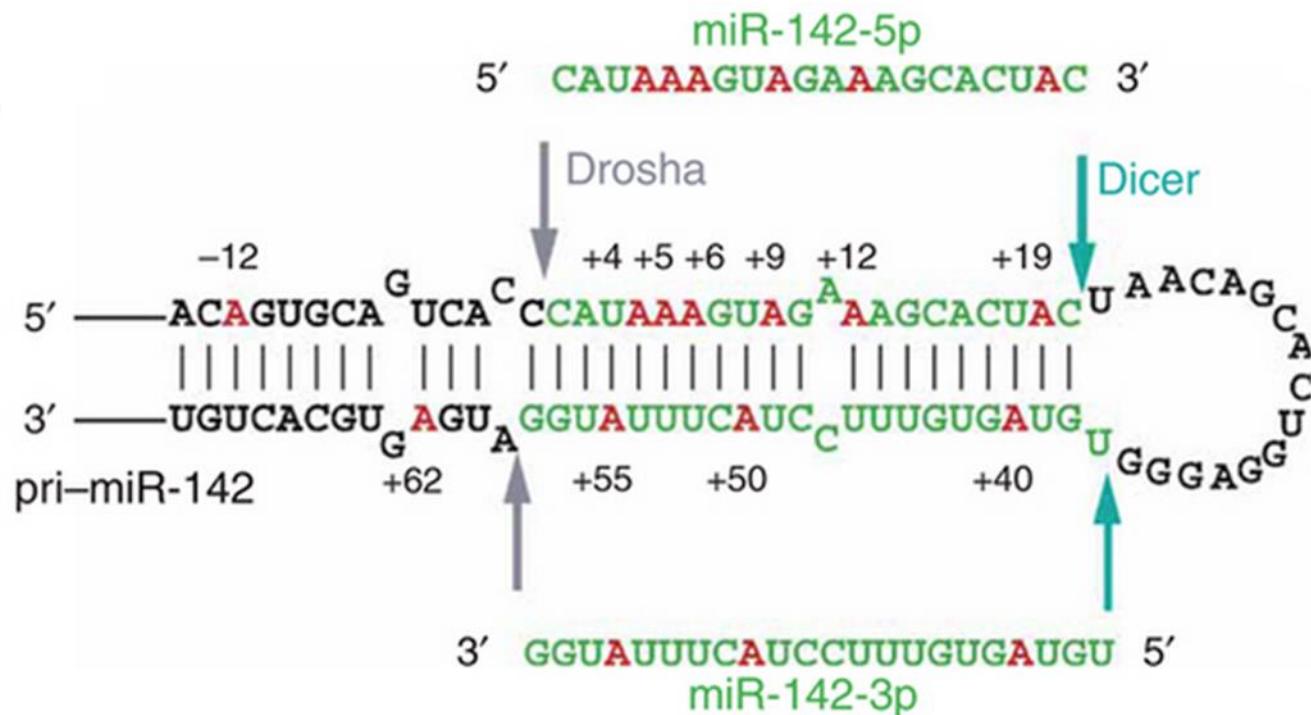
丙肝病毒(HCV), 艾滋病病毒 (HIV)

5.ADAR3由于缺少editing活性，与其他ADAR蛋白竞争RNA，反而起到了dominant negative的作用。

# ADAR1 edits the coding sequence of mRNA



Cell. 1993 Dec 31;75(7):1361-70.



Nat Struct Mol Biol. 2006 January; 13(1): 13–21.

## 研究目的与猜想：

- 1.RNA editing可能是microRNA processing中的一种重要的调控方式。
- 2.通过生物信息学的分析，深入了解ADARB1的序列、结构特征，希望可以利用生物信息学的方法，对ADARB1调控的RNA做一些预测。

# protein sequence alignment

Mouse VS Human ADAR1 protein

Pairwise Alignment Result				
LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
711	3466.5	665/711 (93.5%)	680/711 (95.6%)	10/711 ( 1.4%)

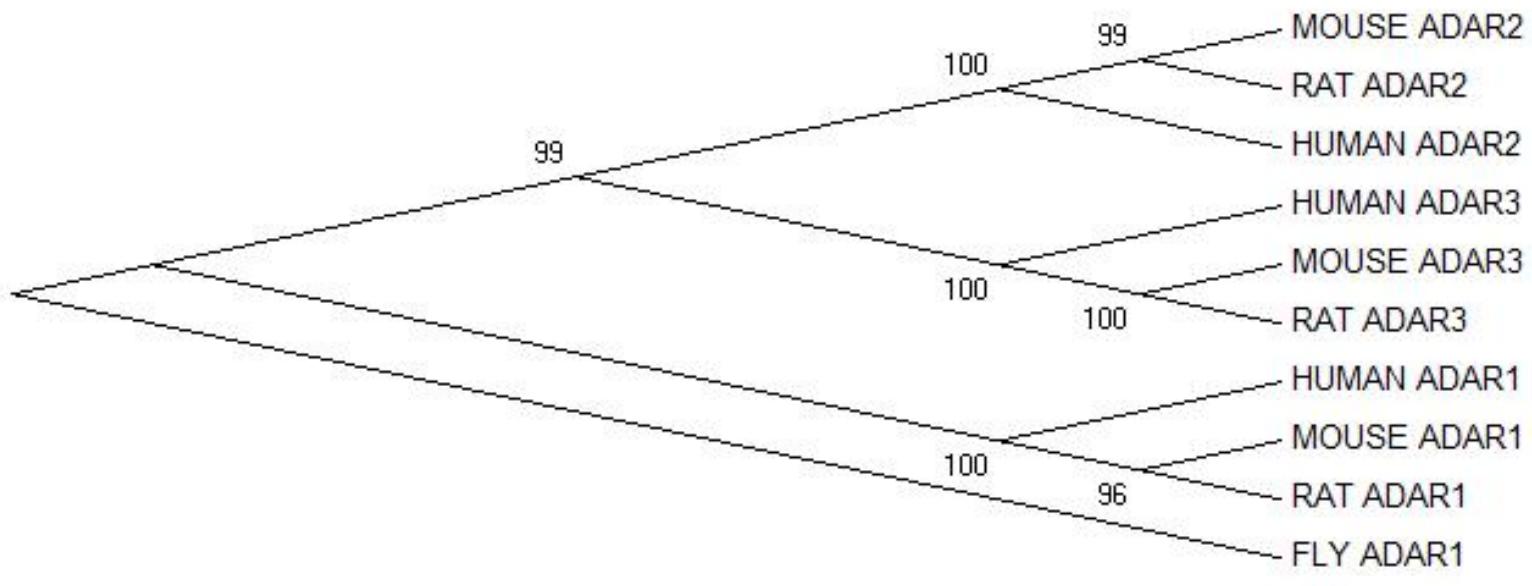
Mouse VS Rat ADAR1 protein

Pairwise Alignment Result				
LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
711	3670.0	704/711 (99.0%)	707/711 (99.4%)	0/711 ( 0.0%)

Human VS Rat ADAR1protein

Pairwise Alignment Result				
LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
711	3465.5	666/711 (93.7%)	680/711 (95.6%)	10/711 ( 1.4%)

# Mega 6.0 phylogeny



# ADAR1

## Sequence annotation (Features)

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier
<b>Molecule processing</b>					
<input type="checkbox"/> Chain	1 – 1226	1226	Double-stranded RNA-specific adenosine deaminase		PRO_0000171774
<b>Regions</b>					
<input type="checkbox"/> Repeat	133 – 202	70	DRADA 1		
<input type="checkbox"/> Repeat	293 – 360	68	DRADA 2		
<input type="checkbox"/> Domain	503 – 571	69	DRBM 1		
<input type="checkbox"/> Domain	614 – 682	69	DRBM 2		
<input type="checkbox"/> Domain	726 – 794	69	DRBM 3		
<input type="checkbox"/> Domain	886 – 1221	336	A to I editase		
<input type="checkbox"/> DNA binding	169 – 195	27			
<b>Sites</b>					
<input type="checkbox"/> Active site	912	1	Proton donor <small>[By similarity]</small>		
<input type="checkbox"/> Metal binding	910	1	Zinc <small>[By similarity]</small>		
<input type="checkbox"/> Metal binding	966	1	Zinc <small>[By similarity]</small>		
<input type="checkbox"/> Metal binding	1036	1	Zinc <small>[By similarity]</small>		

# ADAR2

## Sequence annotation (Features)

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier
<b>Molecule processing</b>					
<input type="checkbox"/> Chain	1 – 741	741	Double-stranded RNA-specific editase 1		PRO_0000171779
<b>Regions</b>					
<input type="checkbox"/> Domain	78 – 144	67	DRBM 1		
<input type="checkbox"/> Domain	231 – 298	68	DRBM 2		
<input type="checkbox"/> Domain	370 – 737	368	A to I editase		
<input type="checkbox"/> Region	83 – 88	6	Interaction with substrate RNA <small>[By similarity]</small>		
<input type="checkbox"/> Region	104 – 105	2	Interaction with substrate RNA <small>[By similarity]</small>		
<input type="checkbox"/> Region	237 – 242	6	Interaction with substrate RNA <small>[By similarity]</small>		
<input type="checkbox"/> Region	259	1	Interaction with substrate RNA <small>[By similarity]</small>		
<b>Sites</b>					
<input type="checkbox"/> Active site	396	1	Proton donor		
<input type="checkbox"/> Metal binding	394	1	Zinc		
<input type="checkbox"/> Metal binding	451	1	Zinc		
<input type="checkbox"/> Metal binding	556	1	Zinc		

# ADAR3

## Sequence annotation (Features)

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier
<b>Molecule processing</b>					
<input type="checkbox"/> Chain	1 – 739	739	Double-stranded RNA-specific editase B2		PRO_0000171782
<b>Regions</b>					
<input type="checkbox"/> Domain	125 – 191	67	DRBM 1		
<input type="checkbox"/> Domain	274 – 341	68	DRBM 2		
<input type="checkbox"/> Domain	408 – 735	328	A to I editase		
<b>Sites</b>					
<input type="checkbox"/> Active site	434	1	Proton donor <a href="#">By similarity</a>		
<input type="checkbox"/> Metal binding	432	1	Zinc <a href="#">By similarity</a>		
<input type="checkbox"/> Metal binding	490	1	Zinc <a href="#">By similarity</a>		
<input type="checkbox"/> Metal binding	555	1	Zinc <a href="#">By similarity</a>		

DRBM (double-strand RNA binding motif)

# DRBM of ADAR

Protein Sequences		
Species/Abbrv	Group Name	
1. Human_ADAR1-DRBM1		I E G I L L Y A F A Q T C E F U M I D S E G H E F * F V V V I N G E F A E A G S V A * D A A M A M I L L -
2. Human_ADAR1-DRBM2		S V I T I L L E C M H L G N S C E F I L L S E G A H E F D Y C V A V G A F S V B A S V A Q M A A P A M A L R -
3. Human_ADAR1-DRBM3		V G G I L L Y A E H G F A A E F I L V D S G I H E F V Y I A V G G W F A V C A H S * S Q D A A A A L V L I -
4. Human_ADAR2-DRBM1		- L I V A L M G L N E I G L Q Y I L L S Q T G V H A L F V M S V V N G V F E - S S G I T A A L H A A A L S F V -
5. Human_ADAR2-DRBM2		S G N V V M I L N E L G L Y D F L S E S G E S H A S F V M S V V V D G F F E - G S G I M L A A A A A A L A A I F -
6. Human_ADAR3-DRBM1		- A I V A L V L H E I G L Q Y I V S I G V H A V F A V A V V N G L F E - S T G I A A M A A L A I S F V -
7. Human_ADAR3-DRBM2		G P V V V L L I L A G L Y V C L A A A A F V M A V V D G F F E - G S G I S N L A G A A A A A A L F -

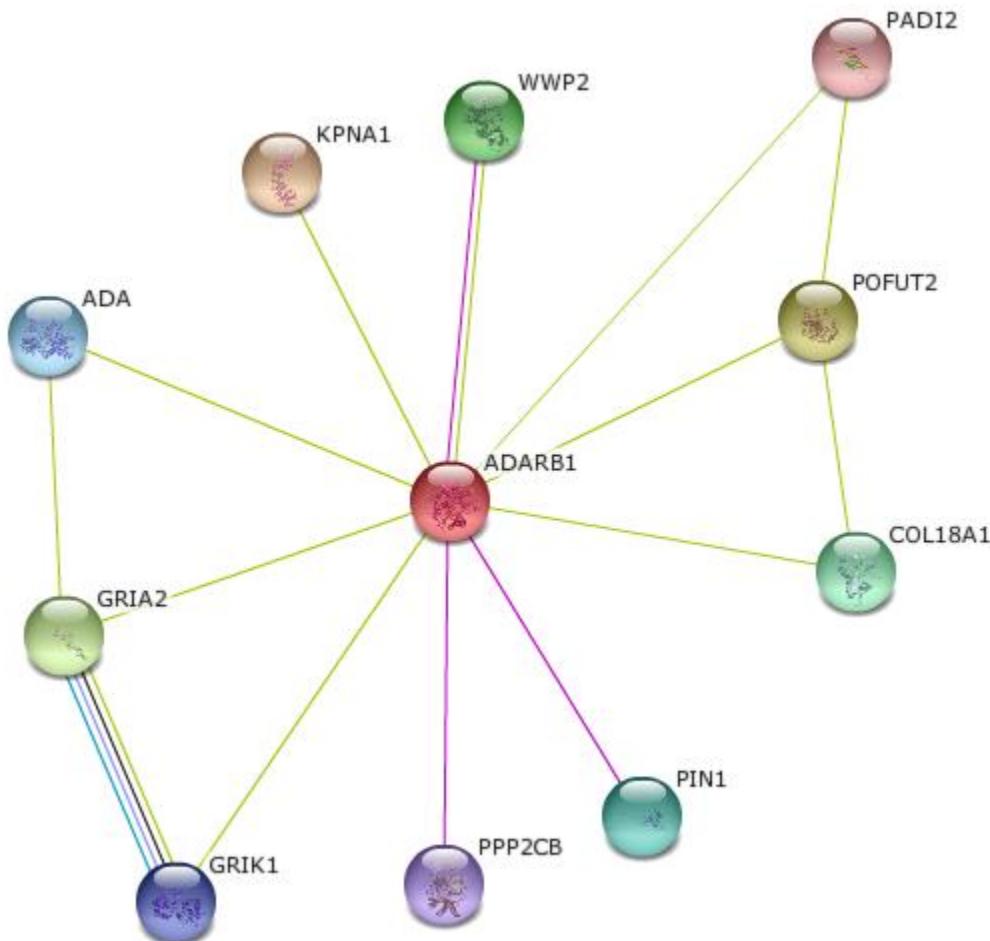


# DRBM of ADARB1 and other RNA binding proteins

Protein Sequences		
Species/Abbrv	Group Name	
1. Human_DICER1-DRBM		- V I S V E L L I M D -- E A F S A -- E T Y D G V V V I V E V V G G F G V G S Y I A S A A A A L I S L -
2. Human_Drosha-DRBM1		- S P V C I L H E Y M D V L I V V Y N F F E -- C E N S E F G A S V I I D G V T Y S S G T A B S I L A V A A A T L E I L I -
3. Human_Drosha-DRBM2		-- Y Q I L H E C L I N R E M G I S I F E -- V V S G N Q S E Y V M A C G H I V G N C I N V G S L A S I I L Q L L H -
4. Human SON-DRBM		- H I V S A L M E I C D I N S Q I F L L V H I S G D H H F L F V L I N G -- A L T I N C M F F L M Y --
5. Human_ADAR2-DRBM1		- L I V A L M G L N E I G L Q Y I L L S Q T -- S V H A L F V M S V V N G Q V F E G S S T V A A L H A A A L S F V -
6. Human_ADAR2-DRBM2		S G N V V M I L N E L G L Y D F L S S -- G E S H A S F V M S V V V D G Q F F E G S S T V A A A A A A L A A I F -



# protein-protein interaction



- WWP2: NEDD4-like E3 ubiquitin-protein ligase WWP2
- PPP2CB: Serine/threonine-protein phosphatase 2A catalytic subunit beta isoform
- PIN1: Telomeric repeat-binding factor 1

大部分基于序列预测，缺乏实验证据的支持。

# Homology Modeling of ADAR2

Workunit: P000005 adarb1\_whole - Overview

1 741  
click on model bars

Models: [1] [2] [3] [4]

Print/Save this page as

**Model Summary** ?

**Model information:**  
Modelled residue range: 74 to 298  
Based on template: [2I3jA] (99.9 Å)  
Sequence Identity [%]: 96.44  
Evalue: 2.98e-109

**Quality information:** [details]  
QMEAN Z-Score: -1.76

**Quaternary structure information:** [details]  
Template (2I3j): MONOMER  
Model: MONOMER

**Ligand information:** [details]  
Ligands in the template: A: 21, C: 13, G: 16, U: 21.  
Ligands in the model: none.

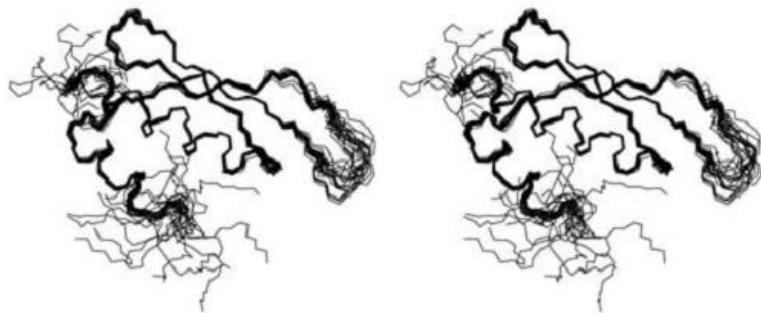
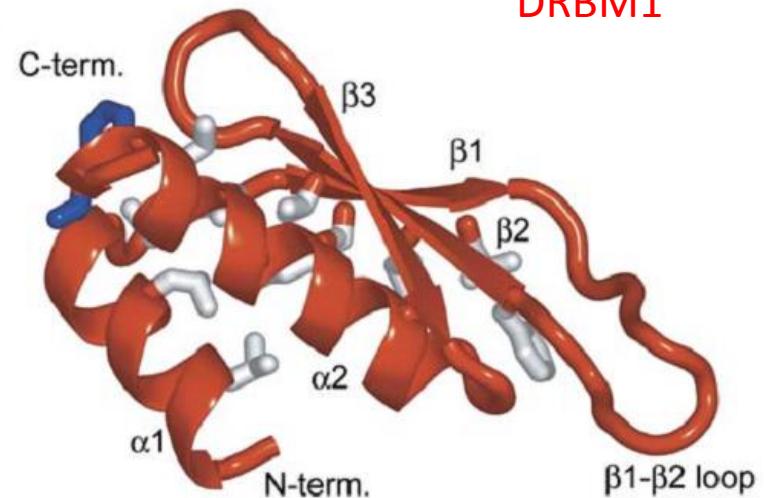
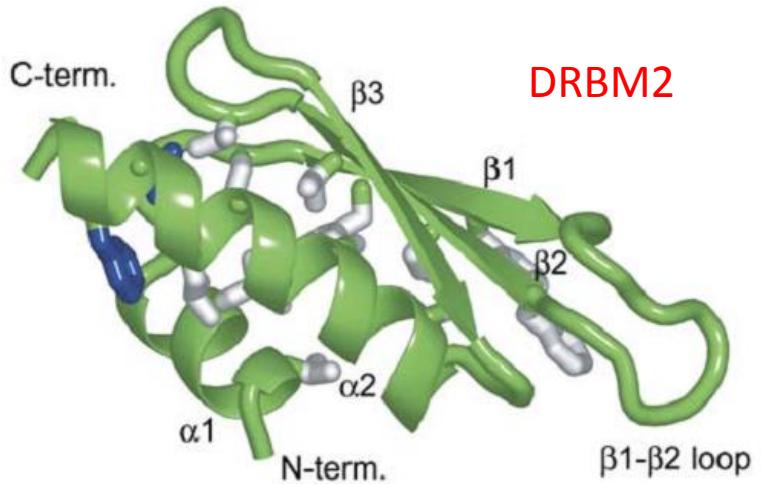
logs: [Templates] [Alignment] [Modeling]  
display model: as [pdb] - as [DeepView project] - in [AstexViewer]  
download model: as [pdb] - as [Deepview project] - as [text]

**Global Model Quality Estimation** ? [+/-]

QMEAN4 global scores: ?		Local scores		
QMEANscore4 ?	Estimated absolute model quality ?	Score components ?	Coloring by residue error ?	Residue error plot ?
0.66	 Z-Score: -1.76 Plot 1: [save png] Plot 2: [save png]	 [save png]		 [save png]
			Coloring (all chains): [save jpg] [save pdb]	Energy profile: ? [save raw scores]

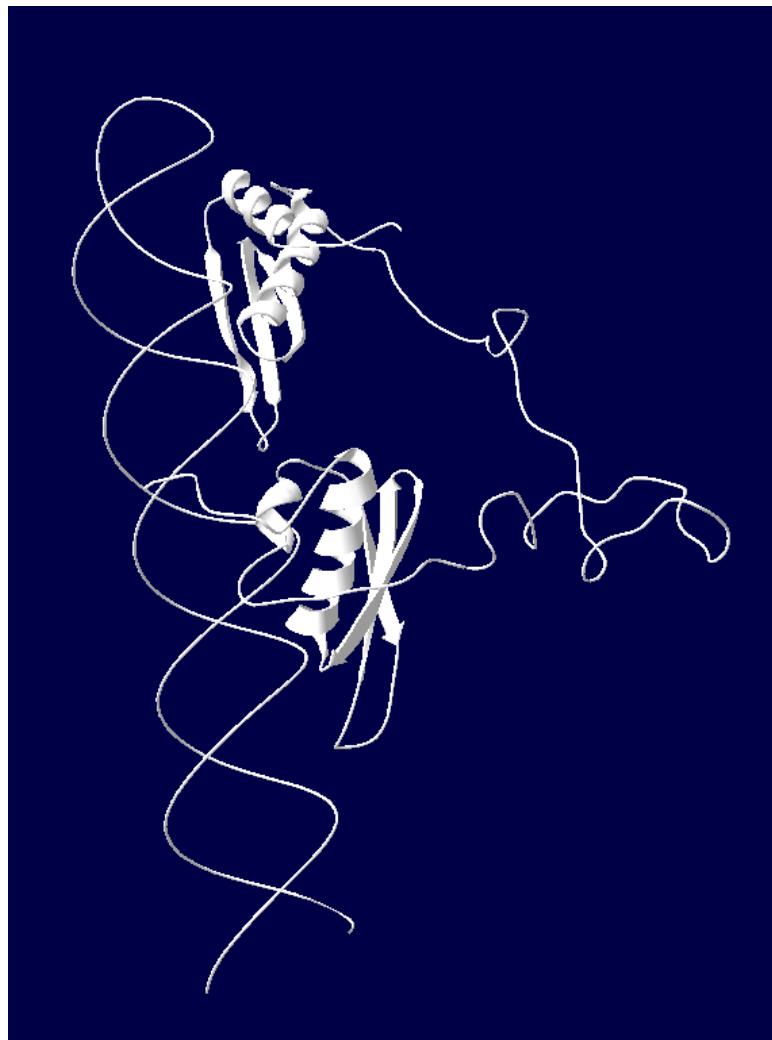
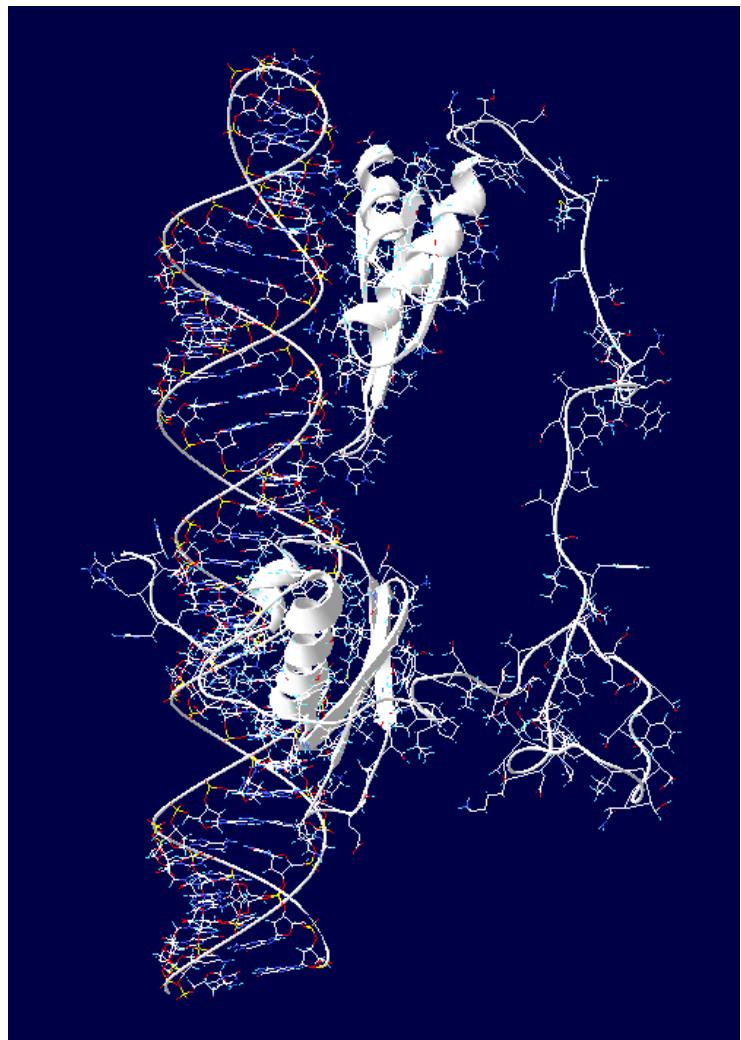
# Search ADAR2 in PDB database

2B7T	2B7V	2L3C	2L2K	2L3J	1ZY7
Structure of ADAR2 dsRBM1	Structure of ADAR2 dsRBM2	Solution structure of ADAR2 dsRBM1 bound to LSL RNA	Solution NMR structure of the R/G STEM LOOP RNA-ADAR2 DSRBM2 Complex	The solution structure of the ADAR2 dsRBM-RNA complex reveals a sequence-specific read out of the minor groove	Crystal structure of the catalytic domain of an adenosine deaminase that acts on RNA (hADAR2) bound to inositol hexakisphosphate (IHP)
NMR			X-RAY		

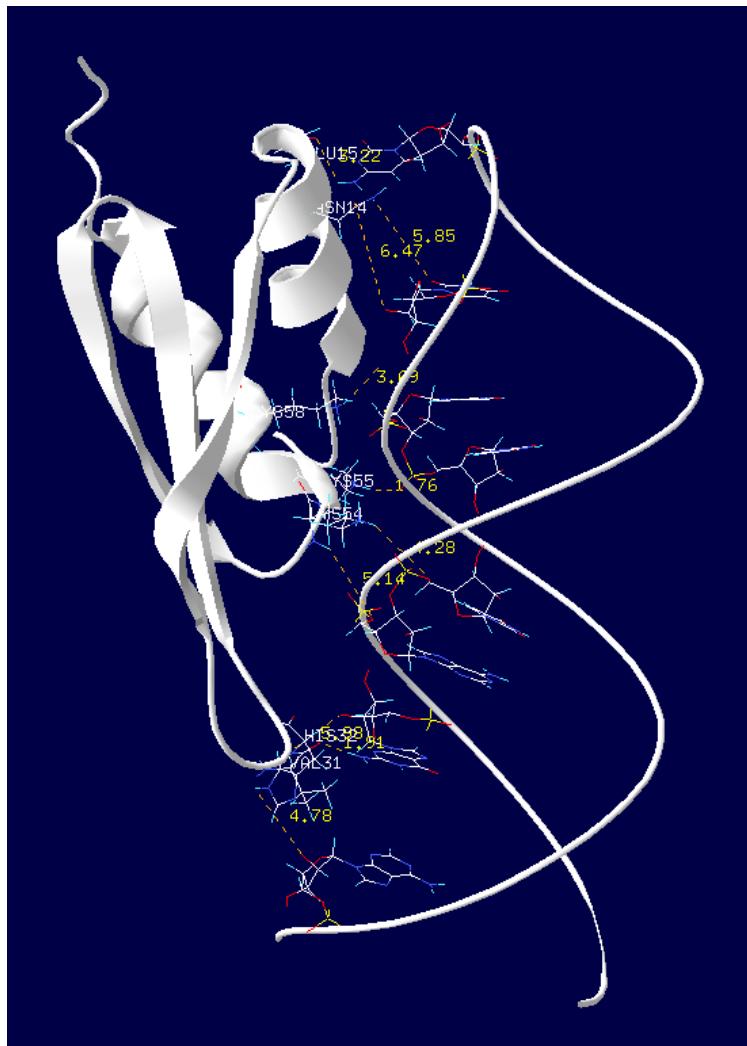
**A****2B7T****B****DRBM1****C****2B7V****D****DRBM2**

Structure. 2006 Feb;14(2):345-55.

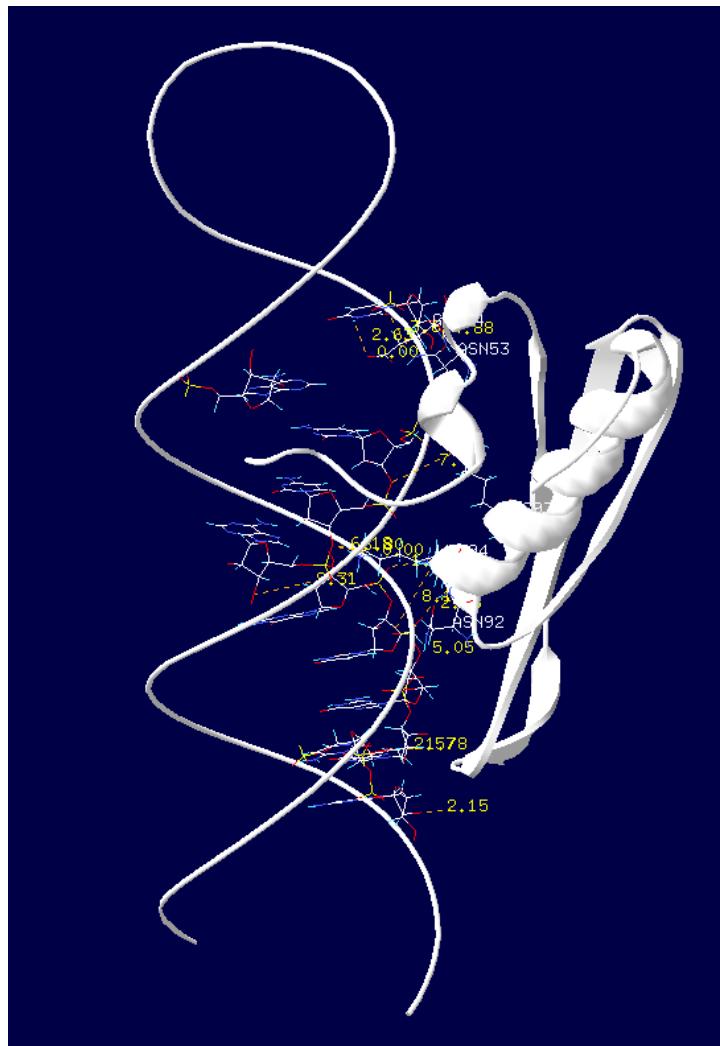
2L3C

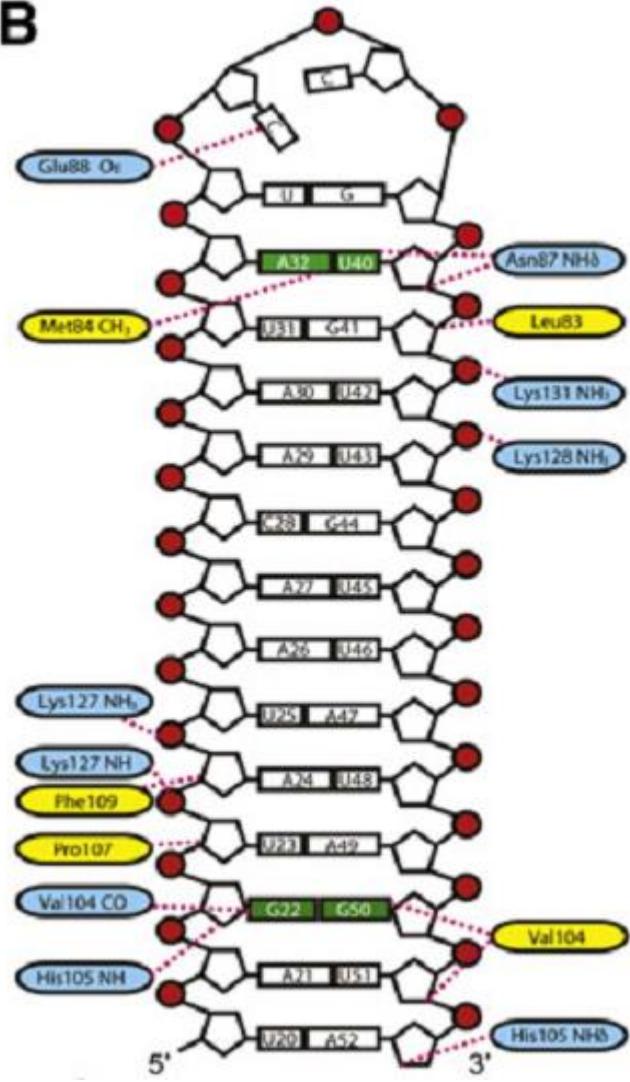
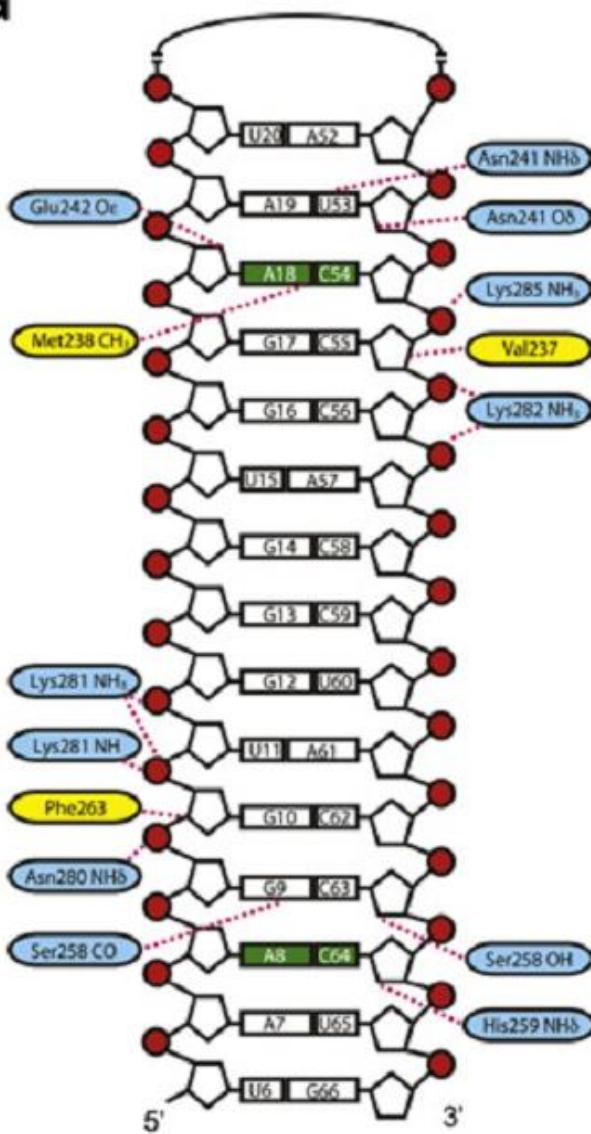


2L3C



2L3k



**B****G**

# Pre-microRNA from miRBase

```
>hsa-mir-3180-2 MI0014215 Homo sapiens miR-3180-2 stem-loop  
GCGACGGGCGGAGCUUCCAGACGCUCCGCCAACGUCGCAUGCGCCCCGGAAAGCGUGG  
>hsa-mir-6875 MI0022722 Homo sapiens miR-6875 stem-loop  
GAGUCUGAGGGACCCAGGACAGGAGAAGGCCUAUGGUGAUUUGCAUUCUUCCUGCCCCUGG  
test.txt (END)
```

## Homo sapiens miR-6875 stem-loop

```

gagu      --- c   a   -   g   cu   g
          cugagg   ga ccagg cagga gaa gc   au g
          |||||   || ||||| ||||| ||||| ||||| ||||| |
          gacucc   cu ggucc guccu cuu cg   ua u
---       uac   c   c   u   a   uu   g

```

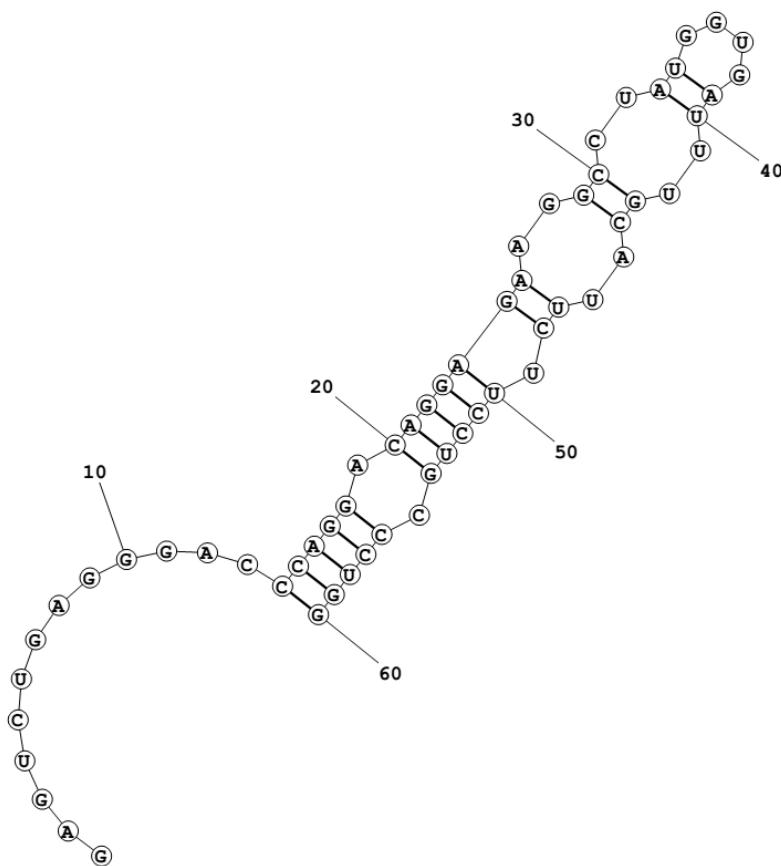
## Homo sapiens miR-3180-2 stem-loop

```

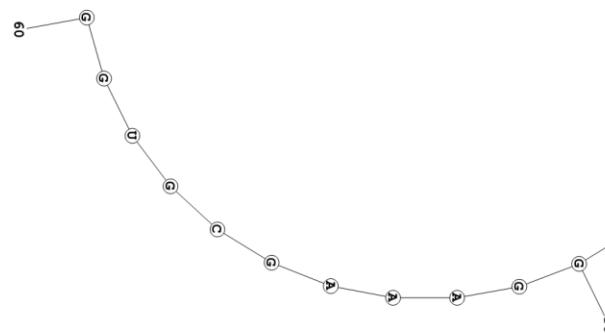
gcgac      a      a   c      ----- c
          gggcgg gcuucc ga  gcuccggccacgu      cg a
          ||||| | | | | ||| | | | | | | | | | |
          cccgcc  cggagg cu  cgaggcggggugcg      gc u
--cgu      c      c   u      aaaggggcccc  g

```

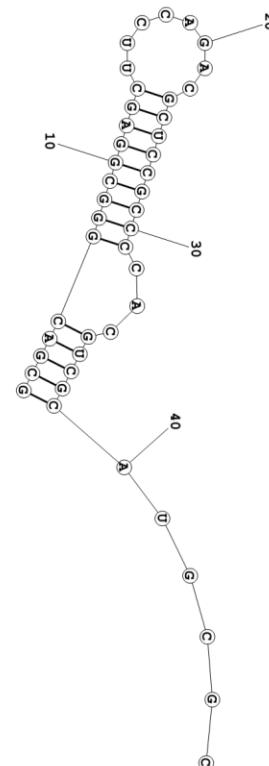
# microRNA Structure Prediction



ENERGY = 5.3 hsa-mir-6875 MI0022722...



ENERGY = 5.7 hsa-mir-3180-2 MI00142...



<http://rna.urmc.rochester.edu/RNAstructure.html>

# 小结与讨论

- ADARB1是一类普遍存在的RNA编辑酶，在物种间的氨基酸序列高度保守。
- 从结构上看，ADARB1上的DRBM符合RNA binding protein的DRBM的普遍结构特征，由 $\alpha\beta\beta\beta\alpha$ 的二级结构组成，但是除了几个比较保守的位点之外，不同的DRBM的氨基酸序列相似性并不高。
- 通过核磁共振对ADARB1的两个DRBM结合dsRNA的结构解析，我们了解到DRBM中的 $\alpha_1$ -helix 和 $\beta_1$ - $\beta_2$  loop是结合RNA的重要部分，它的结合具有一定的序列特异性。
- 根据文献提供的ADARB1结合的RNA序列特征，我们找到ADARB1可能的新的microRNA结合底物（hsa-miR-6875, hsa-miR-3180-2），并预测了它们的RNA二级结构，但仍需进一步三维建模以及实验数据验证（如CLIP-Seq等）。

# 参考文献

1. Many roads to maturity: microRNA biogenesis pathways and their regulation. Winter J, Jung S, Keller S, Gregory RI, Diederichs S. *Nat Cell Biol.* 2009 Mar;11(3):228-34. doi: 10.1038/ncb0309-228.
2. MicroRNA: basic mechanisms and transcriptional regulatory networks for cell fate determination. Fazi F, Nervi C. *Cardiovasc Res.* 2008 Sep 1;79(4):553-61. doi: 10.1093/cvr/cvn151.
3. The many roles of an RNA editor. Keegan LP, Gallo A, O'Connell MA. *Nat Rev Genet.* 2001 Nov;2(11):869-78.
4. RNA editing of AMPA receptor subunit GluR-B: a base-paired intron-exon structure determines position and efficiency. Higuchi M, Single FN, Köhler M, Sommer B, Sprengel R, Seuberg PH. *Cell.* 1993 Dec 31;75(7):1361-70.
5. Modulation of microRNA processing and expression through RNA editing by ADAR deaminases. Yang W, Chendrimada TP, Wang Q, Higuchi M, Seuberg PH, Shiekhattar R, Nishikura K. *Nat Struct Mol Biol.* 2006 Jan;13(1):13-21.

6. Structure and specific RNA binding of ADAR2 double-stranded RNA binding motifs. Stefl R, Xu M, Skrisovska L, Emeson RB, Allain FH. *Structure*. 2006 Feb;14(2):345-55.
7. The solution structure of the ADAR2 dsRBM-RNA complex reveals a sequence-specific readout of the minor groove. Stefl R, Oberstrass FC, Hood JL, Jourdan M, Zimmermann M, Skrisovska L, Maris C, Peng L, Hofr C, Emeson RB, Allain FH. *Cell*. 2010 Oct 15;143(2):225-37. doi: 10.1016/j.cell.2010.09.026.

Thank you!